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THE BERRY SKIN PHENOLIC PROFILE OF *VITIS VINIFERA L* (cv *BARBERA*) AS AFFECTED BY TIME OF BUNCH SUNLIGHT EXPOSURE.

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Abstract

The relations existing among early and late bunch sunlight exposure, berry ripening and the anthocyanin and flavonol profiles were studied on *cv.* Barbera in 2005 in North-West Italy. Early bunch sunlight exposure extended the anthocyanin accumulation phase, decreased the esterified anthocyanin percentage and increased the quercetin-monoglucoside amount. On the contrary, the late bunch sunlight exposure reduced the accumulation of anthocyanins, induced an increase of malvidin-3-glucoside and total acylated compound percentages but it did not change the flavonol contents respect to the control. Besides, early and late bunch sunlight exposures diminished the anthocyanin extractability.

Key words: *Cultivar, temperature, anthocyanins, flavonols, anthocyanin extractability.*

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Résumé

L'essai avait pour objet d'étudier l'effet de l'exposition au rayonnement solaire des grappes sur la maturation du raisin et le profil anthocyanique et flavonolique des pellicules. L'étude à été réalisée en 2005 pour le cépage Barbera, en Italie du nord-ouest. L'exposition à été réalisée en effeuillant complètement la zone des grappes en deux époques: précoce (fermeture de la grappe) et tardive (début véraison). Le comportement des plantes effeuillées a été comparé avec celui de témoins non effeuillés.

Les résultats montrent que l'exposition précoce a prolongé la phase d'accumulation des anthocyanes; elle a aussi déterminé une diminution du pourcentage des formes estérifiées des anthocyanes et une forte augmentation de la teneur en quercétin monoglucoside. Au contraire l'exposition tardive a diminué la teneur en anthocyanes totaux et a causé une augmentation du pourcentage soit de malvidin-3-glucoside soit des anthocyanes estérifiés, mais elle n'a pas eu d'effet sur la teneur en quercétin monoglucoside. L'exposition directe des grappes au rayonnement solaire, soit precoce, soit tardive, a causé une diminution significative de l'extractibilité des anthocyanes.

Introduction

Sunlight bunch exposure intensity can influence ripening; sunlight can directly or indirectly (temperature variations) influence the berry composition and the leaf development. Improved sunlight conditions can influence some light depending biochemical processes in berries. Among these processes, the activity of photoinducible enzymes such as phenylalanine ammonia-lyase *(PAL)* and chalcone synthase, which are responsible for the biosynthesis of polyphenols, can be favoured (Roubelakis-Angelakis and Kliewer, 1986; Dokoozlian and Kliewer, 1996). Direct sunlight exposure can induce a bunch temperature increase and this can negatively influence ripening, inhibiting sugar accumulation, increasing malic acid degradation, reducing berry weight, causing sunburn damages and limiting the anthocyanin biosynthesis (Fitzgerald and Patterson, 1994; Vasconcelos and Castagnoli, 2000; Bergqvist et al., 2001; Spayd et al., 2002). Furthermore, it has been demonstrated that high sunlight exposed berries contain less anthocyanins than shaded ones, showing a significant increase of malvidin-3-glucoside percentage and a decrease of malvidin-3-p-coumaroyl glucoside percentage. Besides, bunch sunlight exposure can influence the flavonol accumulation (Price et al., 1995, Haselgrove et al., 2000; Spayd et al., 2002; Downey et al., 2004). In recent times it has been demonstrated that 30 °C temperature can exert different effects on the anthocyanin biosynthesis, depending on the vine phenological phase (Yamane et al., 2006). Also, it has been shown that the intensity of bunch sunlight exposure can influence the berry flavonol concentration (Price et al., 1995, Haselgrove et al., 2000; Spayd et al., 2002; Downey et al., 2004). Little is known about the influence exerted by light and temperature on the individual anthocyanin and flavonol concentration but it seems very likely that the variation of sunlight bunch exposure intensity can influence the relative percentage of individual phenolics. The aim of this work was to deepen the relationships existing between bunch sunlight exposure on one hand and berry ripening, anthocyanin and flavonol profile and extractability on the other.

Materials and methods

The experiment was performed in 2005 on *Vitis vinifera* cv. Barbera vines planted in a hillside vineyard in North-West Italy. The vineyard planted in 1988 on a 20 % slope, was South exposed and the rows were North/South oriented. Vine spacing was 1.0 x 2.5 m; vines were pruned to a single cane and vertical trained and managed following standard viticultural practices for the cultivar and the region. On the experimental vines (15 vines for three replicates and for each treatment) all the fruit zone leaves were removed in two different time: **early** (17 June, 73 BBCH-code: bunches begin to hang) and **late** (12 August, 81 BBCH-code: berries begin to develop color). The performances of these plants were compared with that of the control vines. During the observation season, main macroclimatic parameters (temperature and rainfall) were measured.

Three samples of 150 berries were collected from 31 August to 5 October (data corresponding to harvest) to evaluate technological ripening and polyphenolic profile. Berry skins of three groups of 10 berries each were separated from the pulp and then extracted at 30 °C for 72 hours in a pH 3.2 ethanol:water buffer, containing 2 g/l or 40 mg/L of $Na_2S_2O_5$, for the determination of total anthocyanins and flavonols and of extractable anthocyanins, respectively (Ummarino *et al.*, 2001). Anthocyanins and flavonols were detected by HPLC (Di Stefano and Cravero, 1991); their quantification (mg/kg of berry fresh weight) was made by the external standard method, the flavonol concentrations were expressed as quercetin dihydrated (Fluka, Buchs, CH) and the anthocyanin concentration as malvidin-3-O-glucoside chloride (Extrasynthèse, F).

Results and discussion

2005 climatic conditions were partially anomalous, as the July-October period temperatures were lower than the averages of the period whereas rainfall was abundant, particularly in September, negatively influencing ripening (Table 1). Detected parameters at harvest are shown in Table 2.

Berry skin total anthocyanin concentration at harvest was significantly higher in early-exposed (EE) bunches than in controls, whereas it was significantly lower in late-exposed (LE) bunches (Table 3). EE bunches did not only show a higher anhocyanin concentration at harvest but also their accumulation phase lasted longer; in LE bunches the anthocyanin biosynthesis was notably slowed down, also in comparison with controls (Fig. 1). As exposed bunch temperature was notably higher than shadowed bunch one (Fig. 2), and as EE bunches were exposed to high temperature longer, it seems possible that the negative effect exerted by temperatures on anthocyanin synthesis (Bergqvist *et al.*, 2001; Spayd *et al.*, 2002) was not univocal. It seems that it depended on the time of intervention, being different if it happened during the early phases of fruit development or later in the season, confirming what it was assessed using a 30 °C temperature (Yamane *et al*., 2006). The results we observed can also be explained admitting that during a not particularly hot and dry seasons, like 2005, the high temperature effects on anthocyanin can be faded by the higher water availability due to rainfall. When anthocyanins were extracted with 2g/L $Na_2S_2O_5$ (Table 3), which can be considered as a total extraction, early leaf-removal caused a reduction of the acylated form percentages whereas late leaf-removal reduced the anthocyanin concentration and increased malvidin-3-O-glucoside percentage, partially confirming previous studies (Spayd *et al.,* 2002; Downey *et al.,* 2004). Leaf-removal timing also influenced the extractable anthocyanin profiles (which were extracted with just 40 mg/L of $Na_2S_2O_5$). As a matter of fact, early leaf-removal caused an increase of cyanidin-3-glucoside percentage and a decrease of acylated form percentage; late leaf- removal caused a decrease of extractable anthocyanins, of delphinidin- and petunidin-glucoside percentages and of total acylated forms whereas it increased malvidin-3-glucoside percentage both in comparison with controls and with early-exposed bunches. The anthocyanin extractability diminished when vines were leaf-removed, independently from time of intervention with the only exception of cyanidin-3-glugoside. Early leaf-removal induced a higher anthocyanin accumulation in absolute terms but this aspect would not probably allow to improve the wine colour; to do that it is probably necessary to vary the maceration and the wine-making technique to obtain a more coloured wine. Thus, being leaf-removal a technique routinely used to better ripening and to increase berry skin colour, the present results make it necessary to revise this cultural practice.

The main flavonols of ‘Barbera’ variety are myricetin-monoglucoside, quercetin-monoglucoside and quercetin-glucoronide. During ripening their amounts in berry skins significantly varied as a function of treatments: in EE berries the flavonol concentration at harvest was higher whereas it was lower in LE ones in comparison with controls. EE bunches showed a significantly higher quercetin concentration (both forms) in comparison with controls whereas LE berries were similar to controls (Figure 3). These results confirm that quercetin biosynthesis or, more in general, flavonol biosynthesis in *Vitis vinifera* berries increases with the increase of fruit sunlight exposure (Price *et al.,* 1995; Ryan *et al.*, 1998; Haselgrove *et al.*, 2000; Spayd *et al.,* 2002; Downey *et al.*, 2004). Besides, it seems evident that the bunch exposition rate during the first stages of berry development, plays an important role in determining the final flavonol concentrations: LE bunches, even though they were sunlight exposed since veraison onwards, did not recover the gap in flavonol concentration in comparison with controls. Berry sunlight exposure during phases I and II of their development exerts a fundamental role in conditioning the flavonol seasonal accumulation, similarly to what it was previously put in evidence for anthocyanins (Dokoozlian and Kliewer, 1996).

Conclusions

The early sunlight exposure of cv Barbera berries in 2005 put in evidence that early leaf-removal (after fruit-set) influenced both anthocyanin and flavonol concentrations and partially modified the individual concentration of the two different classes compounds. Bunch late exposure (at veraison) less influenced the polyphenolic concentration, particularly that of flavonols. Both early and late bunch exposures reduced the anthocyanin extractability, thus the higher anthocyanin concentration we detected would not result in an increase in wine colour, unless extraction procedures are changed. These results let us make the hypothesis that the effects exerted by bunch higher light exposure and high temperatures on polyphenols depend on the bunch phenological phase, that is on when it is exposed to sunlight. Leaf-removal, increasing the fruit-zone sunlight exposure, can also consistently modify the fruit-zone microclimate, conditioning ripening and berry composition. Thus these results are also useful to program leaf-removal timing and techniques.

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Table 1- Meteorological data of Carpeneto (AL) in 2005 (supplied by Regione Piemonte). Tmax = average of maximum temperatures; Tmed = average of medium temperatures; Rtot = total rainfall; GDD = growing degree-days (base 10 °C).

	Tmax	Tmed	Rtot	GDD
April	16.1	11.8	67.4	63
May	23.4	17.9	61.8	246
June	27.5	22.4	7.6	371
July	29.5	23.7	70.8	424
August	27.6	21.8	62.0	366
September	24.0	19.2	111.2	275
October	16.2	13.0	69.6	94
Total			450.4	1839
average '01-'04			433.1	2008

Table 2 - Yield parameters and total soluble solid content (TSS) at harvest in 2005, cv Barbera. In the same column numbers followed by different letters significantly differ for $p \leq 0,05$).

	cluster weight (g)	cluster per vine (n)	yield per vine (kg)	berry weight (g)	TSS (°Brix)
control	214 a	18,1 a	3,9 a	2,76 b	20,8 b
early exposure	194 a	15,6 b	3,0 b	2,72 ab	22,1 a
late exposure	207 a	17,5 a	3,6 ab	2,88 a	21,5 ab

Table 3 - Amounts of anthocyanins (anthoc., mg/kg) and anthocyanin profile (%) of berry skins at harvest in controls and early and late exposed bunches as influenced by the extraction technique; (Df = delphinidin, Cy = cyanidin, Pt = petunidin, Pn = peonidin, Mv = malvidin; 3gl = 3-O-glucoside, Tot. acyl. = total anthocyanin derivative forms). (In the same column within the same extraction technique, numbers followed by different letters significantly differ for $p \leq 0.05$).

extraction technique	treatment	anthoc. (mg/kg)	df (%)	cy (%)	pt (%)	pn (%)	mv (%)	tot. acyl. (%)
total anthocyanins (extraction with **2 g/L** of **$Na_2S_2O_5$)**	control	1425 b	15.0 a	6.3 a	14.2 a	5.8 a	36.9 b	21.8 a
	early	1714 a	15.5 a	6.6 a	14.7 a	6.0 a	36.9 b	20.4 b
	late	1315 c	14.4 a	6.1 a	14.0 a	5.8 a	38.3 a	21.5 a
extractable anthocyanins (extraction with **40 mg/L** of **$Na_2S_2O_5$**)	control	468 a	8.9 a	4.4 b	12.4 a	5.9 a	49.5 b	19.1 a
	early	465 a	8.5 a	5.5 a	12.2 a	6.5 a	50.2 b	17.1 b
	late	356 b	6.3 b	3.6 b	10.7 b	5.5 a	56.1 a	17.8 b
extractability (%)[a]	control	32.8 a	19.5 a	22.9 a	28.5 a	33.2 a	43.8 a	28.8 a
	early	26.5 b	14.6 b	22.1 a	22.0 b	28.9 b	36.1 c	22.3 b
	late	26.9 b	12.0 b	16.1 b	20.7 b	26.1ab	39.2 b	22.4 b

[a] = Extractability was calculated as percentage on the individual compound concentration (mg/kg).

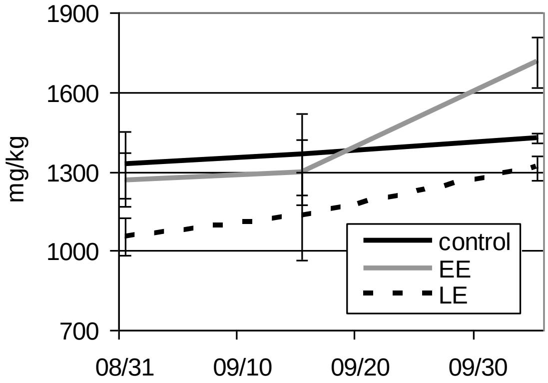

Figure 1 - Seasonal changes in skin anthocyanin total amounts in 2005, cv. Barbera (averages ± standard error); EE = early-exposed vines, LE = late-exposed vines.

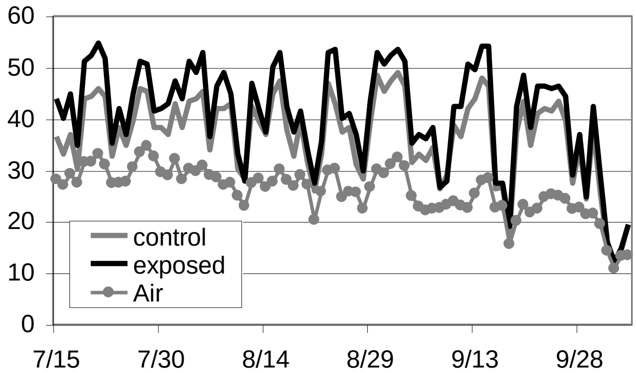

Figure 2. Sub-cuticular temperature (daily maximum temperature) of Barbera berries, detected by thermo-couple (HOBO mod. PAR Sensor S-LIA-M003), in control vines, early- and late exposed vines in comparison with air temperature during 2005 season. Late-exposed berry temperature was the same of control berries until the 12th of August and of exposed berries from that moment onwards.

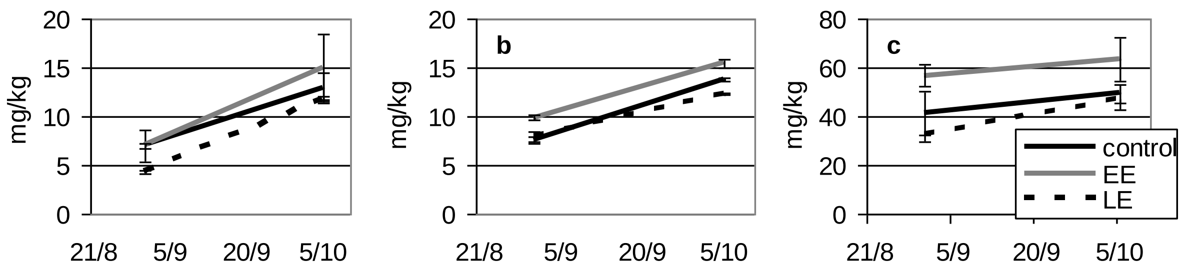

Figure 3 - Seasonal changes in skin flavonol amounts in 2005, cv. Barbera (averages ± standard error). (a): myricetin-monoglucoside, (b): quercetin-glucuronide, (c): quercetin-monoglucoside; EE = early-exposed vines, LE = late-exposed vines.